Experimentally, the laser irradiation power dependency was statistically analyzed for 181 cells with 1Hz periodic exposure to pulse trains, resulting in an irradiation power window from 15 to 30 mW for the pacemaker effects. Higher powers produced constant increments in intracellular calcium. Results correspond with our previous work in non-excitabile cells [1]. We also examined the irradiation phase/frequency dependency of the pacemaker effects by employing a modeling approach based upon known electrophysiology of heart muscle cells. We simulated the pacemaker effects by modifying existing simulation models to include periodic calcium elevations to approximate the laser effects on heart muscle cells. The results show that the pacemaker effects can preferentially occur at certain exposure periods, and that short-term observable pacemaker effects will be dependent on the phases of pulse trains. Assembling these experimental and numerical results will enable sensitive optical controls of the heart muscle cellular beating.

[1] N. Smith, et al., Appl. Phys. Lett., 2001. [2] N. Smith, et al., Opt. Exp., 2008.

2P-001 2次元フーリエ級数による主鎖二面角のエネルギー項を用いたタンパク質系のシミュレーション

Folding simulation using a new backbone-torsion energy term

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Force fields for protein systems, such as AMBER, CHARMM, OPLS, and GROMOS, are widely used for molecular simulations of proteins and peptides. However, it has been shown that these force fields have quite different secondary-structure-forming tendency by the detailed comparisons of these force fields by performing generalized-ensemble simulations of two peptides in explicit solvent model. We need to refine and improve the existing force-field parameters. Among the force-field terms, the torsion-energy term is the most problematic. In some recent proposed force fields, new torsion energy parameters or terms are proposed, such as AMBER ff99SB, ff03, and CHARMM 22/CMAP.

Therefore, we proposed a new backbone-torsion-energy term in the force field for protein systems. This torsion-energy term is represented by a double Fourier series in two variables, the backbone dihedral angles ϕ and ψ . This expression gives a natural representation of the torsion energy in the Ramachandran space. We can then easily control secondary-structure-forming tendencies by modifying the backbone-torsion-energy surface. We tested whether our modifications of the backbone-torsion-energy term resulted in the expected change of secondary-structure-forming tendencies. Moreover, we performed the optimization of the Fourier-coefficient parameters using our optimization method. We then performed folding simulations of some peptides using the optimized Fourier-coefficient parameters. In this paper, we presents the details of these results.

2P-002 高速かつ高精度な van der Waals 相互作用計算スキームを含む QM/MM-MD 計算プログラムの開発

Development of QM/MM molecular dynamics calculation program including accurate and effective description of van der Waals interactions

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Conventional *ab initio* calculations, such as density functional theory (DFT), we face the following difficulties to analyze biological macromolecular systems, i.e. (i) huge size of systems investigated, (ii) an absence of effects of finite temperature, and (iii) a failure of evaluation of van der Waals (vdW) interactions. To overcome (i) and (ii), quantum mechanics / molecular mechanics (QM/MM) molecular dynamics (MD) simulation is a powerful scheme; for the purpose, we have developed an interface program to connect QM (Gamess) and MM (Amber) engines in this study, both of which are highly-parallelized.

As for (iii), LJ potential used in MM calculations are applied also to QM regions; but, to describe interaction energy of stacking, which is crucial for stabilization of biological molecules through vdW interactions, much higher-level *ab initio* calculations, such as CCSD(T) and quantum monte carlo (MC), are still required. However, their computational costs are too large. Accordingly, in this study we have developed a novel algorithm to estimate vdW energy at the CCSD(T) level.

The computational time for our scheme is dramatically reduced by 10^6 fold, when compared with that of the CCSD(T), in spite that accuracy of energies calculated by our new scheme is definitely comparable with that obtained using CCSD(T) calculations. This algorithm has been already implemented in our QM/MM calculation system described above. In this way, three knotty issues in *ab initio* calculations to be overcome are successfully resolved in our system.

2P-003 分子ドッキングおよび QM/MM 分子動力学シミュレーションによる酵素反応機構の理論的解析

Theoretical investigation of enzymatic reaction mechanism by molecular docking and QM/MM-MD simulations

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Aminoacyl-tRNA synthetases play a critical role in decoding genetic information through catalyzing attachment of their cognate amino acid to 3'-end of the specific tRNA. The fidelity of translation is assured by their strict discrimination of the cognate amino acids from non-cognate ones. However, in several cases, e.g., the leucine system, leucyl-tRNA synthetases (LeuRS) have difficulties in the strict discrimination due to existence of similar amino acids, isoleucine and valine.

Thus the LeuRS produces mis-aminoacylated tRNAs. To avoid this error, the enzyme has editing function to hydrolyze the mis-products by themselves. Although several crystal structures of LeuRS in complex with tRNA^{Leu} are available, reaction mechanisms of the editing have not been clarified due to the reasons: (i) mis-aminoacylated tRNAs are not included (ii) nucleophile has not yet been identified.

Here, we performed molecular docking of the LeuRS/tRNA^{Leu} complex and an incognate amino acid, Val, by using a novel algorithm developed by us, referred to as the "Fully Solvated Dynamical Docking" (FSDD). Thereby, we have successfully identified ordered water molecules in the active site, and it has been found that one of such waters is located at a proper position as nucleophile. Then, using the modeled LeuRS/Val-tRNA^{Leu} complex, we adopted a QM/MM program developed by us, which couples QM and MM engines. Thereby, it has been found that LUMO is located on the reaction point of the substrate, suggesting that the water identified attacks LUMO as nucleophile.

2P-004 電圧感受型モータータンパク質プレスティンの電子顕微鏡を用いた構造解析

Single particle analysis of the motor protein "prestin"

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Mammalian ear has specialized function in collecting and amplifying sound signals. Prestin is a transmembrane motor protein localized at the outer hair cells (OHCs) of the inner ear, first identified using subtractive cloning between the motile OHC cDNA library and the non-motile inner hair cells (Zheng, 2000). Voltage-dependent conformational changes in prestin generate changes in the length of OHCs. A positive feedback loop generates synchronous force by sensing the vibration within the organ of Corti, which amplifies sound signals more than 100 times. The change in the length of OHC cells occurs in microseconds (as fast as 20 kHz), the fastest movement among known motor proteins. Actually, disruption of prestin is considered as a cause of hearing loss and subjective tinnitus by administration of large doses of aspirin. To understand the generation of force and its regulation mechanisms, we purified recombinant prestin protein from baculovirus-infected Sf9 cells, observed the structure of negatively stained molecules using EM, and reconstructed the 3D structure by single particle analysis. Biochemical analyses show the prestin is 4-fold symmetry. 13,149 particles were selected from negatively stained prestin. The three-dimensional structure reconstructed at 2 nm resolution revealed a bullet-shaped molecule with dimensions of 11.5 nm in height and 7.7 nm in side length. We also postulate low density space inside the molecule where the bound Cl⁻ ions moves within the molecules.

2P-005 Grb2 と T 細胞補助レセプター-CD28 細胞質内ドメインとの分子間相互作用

Molecular interaction of Grb2 with the cytoplasmic domain of T cell costimulatory receptor CD28

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In addition to the interaction between the T cell receptor and specific peptide/MHC complexes, the costimulatory interaction between CD28 molecules expressed on T cells and B7 molecules on antigen presenting cells provides activating signals to T cells. A number of signaling molecules such as phosphatidylinositol 3-kinase and Grb2 are involved in CD28-mediated costimulatory signals. The cytoplasmic domain of CD28 has 41 amino acid residues, and contains both SH2 and SH3 binding motifs. Human Grb2 has 217 amino acid residues, and comprises a central SH2 domain flanked by two SH3 domains. The Grb2 SH2 binding to the site in CD28, pYMN, is critical, while its SH3 binding to the site(s) in CD28, PXXP, is still controversial. To determine the contribution of Grb2 SH3, we overexpressed Grb2 and its SH2 domain in *E. coli* with N-terminal GST-tag, and analyzed their interactions with the chemically synthesized cytoplasmic domain of CD28 using surface plasmon resonance biosensor. The results showed that the binding affinity of intact Grb2, SH3-SH2-SH3, was about three-times higher than that of Grb2 SH2, indicating that Grb2 SH3 also contributes to the CD28 binding. In contrast, the binding affinity of intact Grb2 to the mutant CD28, in which Pro residues in the SH3 binding sites are mutated to Ala, was similar to that to the wild-type CD28. To confirm the binding mode and determine the three-dimensional structure of Grb2-CD28 complex, the ¹⁵N uniformly labeled Grb2 samples were prepared, and the NMR experiments are in progress.

2P-006 Structural studies of the VHS domain of human STAM1 protein

Structural studies of the VHS domain of human STAM1 protein

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VHS (Vps27/Hrs/Stam) domains consist of ~150 amino acids and are found in at least 60 proteins. In mammals, the VHS domain is present in eight proteins, including Golgilocalizing/ γ -adaplin ear homology domain/ ADP-ribosylation